

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

Claims 1-2 (canceled)

3. (previously presented) An isolated conjugate comprising

(a) at least one

(i) ubiquitin or

(ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and

(b) a protein, wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, and fragments and derivatives thereof;

wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins, and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue and an internal Lys residue.

4. (previously presented) The conjugate of claim 3, wherein said conjugate is immobilized on a support and/or linked to a label.

5. (withdrawn) A method for producing a conjugate comprising

(a) at least one

(i) ubiquitin or

(ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and

(b) a protein, wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, tau and fragments and derivatives thereof; comprising:

A) forming a mixture comprising a vector containing a clone coding for said protein, or said fragment or derivative thereof, an in vitro transcription/translation system, an N-rule ubiquitylation system and, optionally, a proteasome inhibitor; and

B) incubating said mixture to allow production of said conjugate;

wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins, and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue and an internal Lys residue.

6. (withdrawn and currently amended) The method of claim 5, further comprising:

eC) isolating said conjugate.

7. (withdrawn) The method of claim 6, wherein said isolating is done by binding to an antibody specific to a poly-ubiquitin chain.

8. (withdrawn) The method of claim 6, wherein said isolating is done by binding to an antibody specific for said protein.

Claim 9 (canceled)

10. (withdrawn) An isolated activated fragment of a protein, said fragment having an exposed N-degron, wherein said protein has a hidden N-degron and is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof.

Claims 11-14 (canceled)

15. (withdrawn) A method of producing an activated fragment of a protein having an exposed N-degron, wherein said protein is selected from the group consisting of

apratxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof, comprising:

- a) forming a mixture comprising said protein and a protease which cleaves said protein to form said activated fragment; and
- b) incubating said mixture to allow production of said activated fragment.

16. (withdrawn) The method of claim 15, further comprising:

- c) isolating said activated fragment.

17. (currently amended) A composition comprising a conjugate comprised of

(a) at least one

(i) ubiquitin or

(ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and

(b) a protein, wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6 and fragments and derivatives thereof; [[:]]

wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins, [[:]] said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue plus an internal Lys residue, [[:]] and said conjugate is immobilized on a support and/or linked to a label.

Claims 18-34 (canceled)

35. (withdrawn) A method for identifying one or more active compounds that modulate N-end rule dependent ubiquitylation of a protein selected from the group comprising aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, comprising:

- a) forming a mixture comprising said protein or a fragment or derivative thereof, an N-rule ubiquitylation system, one or more candidate compounds and, optionally, a proteosome system;
- b) measuring N-end rule ubiquitylation and/or proteosome-mediated degradation of said protein or a fragment or derivative thereof; and
- c) identifying one or more compounds that modulate the rate of ubiquitylation or degradation.

36. (withdrawn) The method of claim 35, wherein said protein or a fragment or derivative thereof includes a pro-N-degron and said mixture of step a) further includes a protease which exposes said N-degron.

37. (withdrawn) The method of claim 35, wherein said protein or a fragment or derivative thereof is an activated fragment of said protein having an exposed N-degron.

38. (withdrawn) The method of claim 35, wherein said active compound modulates activity of an E1 ligase, E2 ligase, E3 ligase, a protease that exposes said N-degron, or a combination thereof.

39. (withdrawn) The method of claim 35, wherein said active compound modulates activity of an E1 ligase, E2 ligase and/or E3 ligase, or a combination thereof.

40. (withdrawn) A method for determining the mechanism of a compound that affects N-end rule ubiquitylation, comprising:

- a) performing the identifying method of claim 36;
- b) repeating said identifying method, except that said mixture further comprises an inhibitor of N-end rule ubiquitylation or said protein is replaced with a pre-activated fragment of said protein having said exposed N-degron; and
- c) determining whether said compound is specific for said protease, and/or said N-end rule ubiquitylation system.

41. (withdrawn) A method for determining the mechanism of a compound that affects N-end rule ubiquitylation, comprising:

- a) performing the identifying method of claim 35;
- b) repeating said identifying method, except that said mixture further comprises an additional modulator of Type I, Type II and/or Type III N-end rule ubiquitylation; and
- c) determining where said compound affects Type I, Type II and/or Type III N-end rule ubiquitylation.

42. (withdrawn) A method of making a pharmaceutical formulation containing one or more active compounds which modulate N-end rule ubiquitylation of a protein selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, comprising:

- a) forming a mixture comprising said protein, or an activated fragment of said protein having an exposed N-degron, an N-rule ubiquitylation system, one or more candidate compounds and, optionally, a proteosome system;
- b) detecting N-end rule ubiquitylation and/or proteosome-mediated degradation of said protein;
- c) identifying one or more active compounds from said one or more candidate compounds; and
- d) incorporating at least one of said one or more active compounds into a pharmaceutical formulation comprising said at least one active compound and suitable carrier.

43. (withdrawn) The method of claim 42, wherein said one or more active compounds are inhibitors of N-end rule ubiquitylation.

44. (withdrawn) The method of claim 42, wherein said one or more active compounds are promoters of N-end rule ubiquitylation.

45. (withdrawn) The method of claim 42, wherein said one or more active compounds are naturally occurring.

46. (withdrawn) The method of claim 42, wherein said one or more candidate compounds are selected from a compound library.

47. (withdrawn) The method of claim 42, wherein said one or more candidate compounds are selected from a compound library of FDA approved drugs.

Claims 48-56 (canceled)

57. (currently amended) An isolated conjugate comprising

(a) at least one

(i) ubiquitin or

(ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; [;]] and

(b) a recombinant protein, [;]] wherein said recombinant protein is selected from the group consisting of tau, and fragments and derivatives thereof; [;]]

wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins, [;]] and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue plus an internal Lys residue.

58. (previously presented) The conjugate of claim 57, wherein said conjugate is immobilized on a support and/or linked to a label.

59. (currently amended) A composition comprising a conjugate comprised of

(a) at least one

(i) ubiquitin or

(ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and

(b) a protein, wherein said protein is selected from the group consisting of tau, and fragments and derivatives thereof;

wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within tau₁ [[:]] said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue plus an internal Lys residue₁ [[:]] and said conjugate is immobilized on a support and/or linked to a label.

60. (previously presented) The conjugate of claim 3, wherein said fragment is an activated fragment of a protein, said fragment having an exposed N-degron.

61. (previously presented) The conjugate of claim 60, wherein said activated fragment is immobilized on a support and/or linked to a label.

62. (previously presented) The conjugate of claim 57, wherein said fragment is an activated fragment of a protein, said fragment having an exposed N-degron.

63. (previously presented) The conjugate of claim 62, wherein said activated fragment is immobilized on a support and/or linked to a label.

64. (withdrawn) The method of claim 6, wherein said isolating is done by binding to an said molecule, which is employed for purification or visualization.

65. (currently amended) A conjugate comprising

(a) at least one

(i) ubiquitin or

(ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization₁ [[:]] and

(b) a protein;

said conjugate made by process comprising:

A) forming a mixture comprising

(i) a vector containing a clone coding for said protein, wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6 and tau, and fragments and derivatives thereof; and said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins, and said fragment and derivative comprises a destabilizing N-terminal residue and an internal Lys residue;

(ii) an in vitro transcription/translation system;

(iii) an N-rule ubiquitylation system; and

(iv) optionally, a proteasome inhibitor; and

B) incubating said mixture to produce said conjugate.

66. (previously presented) The conjugate of claim 65, wherein said conjugate is immobilized on a support and/or linked to a label.